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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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DRINKER BIDDLE & REATH (DC)
1500 K STREET, N.W.
SUITE 1100
WASHINGTON, DC 20005-1209

EXAMINER	
SCHNIZER, RICHARD A	
ART UNIT	PAPER NUMBER
1635	
MAIL DATE	DELIVERY MODE
09/30/2009	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/594,772	OCHIAI ET AL.	
	Examiner	Art Unit	
	Richard Schnizer	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 September 2009*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1 and 3-6* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1 and 3-6* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/8/09 has been entered.

Claims 7-11 were canceled by amendment on 8/6/09.

Claims 1 and 3-6 remain pending and are under consideration.

The Declaration under 37 CFR 1.132 of Masako Fukushi-Mizutani was received on 9/8/09 and was considered.

Priority

On 9/8/09 the Office received a certified translation of the foreign priority document, JAPAN 2004-107512, however, the translation only included odd-numbered pages. In the event that the explanation for this is that a two-sided document was supplied to the Office, and only one side of each sheet was scanned, Applicant is advised that 37 CFR 1.52(a)(1)(iii) requires that all papers, other than drawings, that are submitted on paper or by facsimile transmission, and are to become a part of the permanent United States Patent and Trademark Office records in the file of a patent application must be printed on only one side of each sheet of paper. Because the document filed on 9/8/09 is not a complete copy of a translation of the foreign priority

document, the effective filing date of the instant claims remains no earlier than the filing date of PCT/JP2005/05786, i.e. 3/26/05. See MPEP § 201.15.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3, 4, and 6 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Certik et al (Trends in Biotechnology 16(12): 500-505, 1998, of record), Ueda (J. Neurogenetics 15(3-4): 193-204, 2001, of record), and Mackenzie et al (App. Env. Microbiol. 66(1): 4655-4661, 2000, of record).

Certik taught that there was an increasing demand for biologically active polyunsaturated fatty acids (PUFAs) and that oleaginous filamentous fungi possess several advantages as a source for PUFAs. In particular, *Mortierella alpina* is disclosed as one of the best producers of various types of PUFA, and is a strain which has several advantages including: it is a highly oleaginous strain; its lipogenesis is simply regulated; it is one of the most well-studied microorganisms producing PUFAs; the strain is able to incorporate and transform exogenous fatty acids; it is amenable to molecular-genetic study; and the strain can be used in an industrial scale. Certik also disclosed the use of such fungi for producing lipids, wherein fatty acid desaturase activity was decreased by mutation or by use of specific enzyme inhibitors. See

abstract, paragraph bridging left and right columns on page 500; paragraph bridging pages 500 and 501; page 501, left column, first full paragraph; and Fig. 1 on page 501. More specifically, Certik noted that mutants with defective desaturases such as Δ^5, Δ^6, Δ^9, Δ^{12}, and ω^3 are worthwhile as producers of useful PUFAs, and for providing valuable information on PUFA biosynthesis in *M. alpina*. Page 501, left column, last paragraph.

Ueda taught that RNAi provided a means of selectively inhibiting expression of genes of choice that was conserved across a wide variety of organisms including plants animals and fungi. Methods include stably transfecting target organisms with heritable expression constructs encoding RNAi agents. See abstract; page 195, second full paragraph; Fig. 1B on page 196; last paragraph on page 197; and second full paragraph on page 199.

Mackenzie et al (App. Env. Microbiol. 66(1): 4655-4661, 2000) taught means for delivering genetic material to *Mortierella* for stable expression of genes of interest. See abstract, and "Vector construction and transformation of *M. alpina"* at page 4656.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use RNAi to inhibit the activity of any of the Δ^5, Δ^6, Δ^9, Δ^{12}, or ω^3 desaturases of *M. alpina*. One would have been motivated to inhibit these enzymes because Certik indicated that strains defective in these enzymes were useful as producers of PUFAs as well as for providing valuable information on PUFA biosynthesis. It would have been obvious to use siRNA to suppress expression of the genes because use of this method allows one to specifically and selectively target any desaturase gene of interest for which target sequence information was available,

obviating the need to screen for randomly occurring mutants. Further, one would have had a reasonable expectation of success in view of the fact that RNA interference was known to function in fungi (see Ueda) , and in view of the availability of vectors and techniques for establishing stable expression of heterologous genes in *M. alpina* (see Mackenzie).

Claims 1 and 3-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Takeno et al (Appl. Microbiol. Biotechnol. 65: 419-425, 2004) as applied to claim 1 above, and further in view of Ueda (J. Neurogenetics 15(3-4): 193-204, 2001, of record) and Mackenzie et al (App. Env. Microbiol. 66(1): 4655-4661, 2000, of record).

Takeno disclosed the establishment of a microprojectile bombardment-based transfection system of M. alpina, and the establishment of stable transfectants. See abstract. The authors also stated that they "have aimed to overexpress or destroy a gene involved in PUFA biosynthesis". Accordingly it would have been obvious to one of ordinary skill in the art at the time of the invention to have used the method of Takeno to suppress the expression of a PUFA biosynthetic gene by destroying the gene, as suggested by Takeno.

Takeno did not disclose the use of RNAi.

Ueda taught that RNAi provided a means of selectively inhibiting expression of genes of choice that was conserved across a wide variety of organisms including plants, animals, and fungi. Methods include transfecting target organisms with heritable expression constructs encoding RNAi agents. See abstract; page 195, second full

paragraph; Fig. 1B on page 196; last paragraph on page 197; and second full paragraph on page 199.

It would have been obvious to one of ordinary skill in the art at the time of the invention to use siRNA to inhibit expression of a PUFA biosynthesis gene. One of ordinary skill appreciates that genetic methods for physically destroying a gene require recombination events at a precise site, i.e. the site of the gene of interest. However, Mackenzie taught that it was difficult to obtain stable, chromosomally-integrated transformants in *M. alpina*, and that to do so it was necessary to take advantage of the large number of repetitive rDNA sites in the *M. alpina* genome by targeting them for homologous recombination. See page 4655, right column, second sentence of first full paragraph. *M. alpina* contains 150-200 tandemly repeated copies of the rDNA locus per haploid genome. Use of vectors designed to integrate at rDNA loci increases the probability of obtaining stable integrants. See page 4658, left column, first full paragraph, and right column, lines 3-9. Thus one of ordinary skill would conclude that there would be a greater expectation of success in suppressing expression of a target gene if one used siRNA expression vectors targeted to integrate in *M. alpina* rDNA, than if one sought to knock out a specific *M. alpina* gene by homologous recombination. Thus the invention as a whole was prima facie obvious.

Claim 5 stands rejected under 35 U.S.C. 103(a) as being unpatentable over Certik et al (Trends in Biotechnology 16(12): 500-505, 1998, of record), Ueda (J. Neurogenetics 15(3-4): 193-204, 2001, of record), and Mackenzie et al (App. Env.

Microbiol. 66(1): 4655-4661, 2000, of record) as applied to claims 1, 3, 4, and 6 above, and further in view of White et al (US 6,939,704).

The teachings of Certik, Ueda, and Mackenzie are discussed above and can be combined to render obvious methods of suppressing the expression of a PUFA biosynthetic gene in *M. alpina* using RNAi methodology.

These references did not teach gene delivery by electroporation or particle bombardment.

White taught that filamentous fungi could be transfected by several methods including calcium chloride treatment of protoplasts, electroporation, and particle bombardment. See column 12, lines 22-31

It would have been obvious to one of ordinary skill in the art at the time of the invention to use any of calcium chloride treatment of protoplasts, electroporation, or particle bombardment to deliver nucleic acids to M. alpina, because these techniques were suggested for use with filamentous fungi.

Thus the invention as a whole was prima facie obvious.

Claims 1, 3, 4, and 6 stand rejected under 35 U.S.C. 103(a) as being unpatentable over Certik et al (Trends in Biotechnology 16(12): 500-505, 1998, of record), Ueda (J. Neurogenetics 15(3-4): 193-204, 2001, of record), Mackenzie et al (App. Env. Microbiol. 66(1): 4655-4661, 2000, of record), and Parker-Barnes et al (Proc. Nat. Acad. Sci. USA 97(15): 8284-8289, 2000, of record).

The teachings of Certik, Ueda, and Mackenzie are discussed above and can be combined to render obvious methods of suppressing the expression of a PUFA biosynthetic gene in *M. alpina* using RNAi methodology. Certik also disclosed that fatty acid elongase was required in PUFA biosynthesis.

Parker-Barnes discovered a gene encoding a fatty acid elongase gene from *M. Alpina*.

It would have been obvious to one of ordinary skill in the art at the time of the invention to suppress expression of the M. alpina elongase gene of Parker-Barnes using RNAi methodology. One would have been motivated to do so in order to evaluate the function of the gene and its interactions with other genes in the fatty acid biosynthesis pathway. See e.g. Ueda, abstract.

Thus the invention as a whole was prima facie obvious.

Response to Arguments

Applicant's arguments, and the Declaration of Masako Fukushi-Mizutani, filed 9/8/09 have been fully considered but they are not persuasive.

Applicant argues that the information at the time of filing would not have provided one of skill with a reasonable expectation of success that the claimed method would have worked.

First, Applicant asserts that it was not possible at the time of the invention to predict the levels at which the precursors in the relevant biosynthetic pathway would have accumulated, relying for support on paragraph 16 of the Declaration. This is

unpersuasive because Applicant is arguing limitations that are not in the claims. The claims require only suppressing expression of one of the recited enzymes. There are no limitations regarding the extent of the suppression, or the accumulation of any pathway intermediates.

Second, Applicant asserts that there are two Δ6 fatty acid desaturase genes in *Mortierella*, and so it was unpredictable at the time whether or not both genes could be co-suppressed using a construct based on the sequence of only one of the two genes (relying for support on the Declaration at paragraphs 23-26). This is unpersuasive because Applicant is arguing limitations that are not in the claims. The claims do not require cosuppression per se of any gene. The claims recite RNAi and cosuppression steps as alternatives. Even if the claims did require co-suppression, they do not require cosuppression of two genes. Further, the Declaration provides no objective evidence that two genes were suppressed. The fact that *Mortierella* comprises two different Δ6 fatty acid desaturase genes does not mean that both genes would be expected to be expressed under the conditions of the experiment, or that both gene products have similar specific activities. If the second, assumedly non-homologous gene, was not expressed under the culture conditions of the experiment, or it encoded a product that has a much lower specific activity than the enzyme preceding it in the pathway, one would reasonably expect its substrate to accumulate.

Third, Applicant asserts that one would not have reasonably expected that suppression of genes encoding desaturases would have worked by either an RNAi method or a cosuppression method, relying for support on the Declaration at

paragraphs 14-26. These Declaration paragraphs provide objective evidence that one could transfect *A. mortierella* fungi with an expression vector encoding a desaturase gene of interest and select transformants that subsequently underexpress the desaturase gene product relative to wild type, leading to accumulation of the desaturase substrate. The specification at pages 34-47 provides objective evidence that RNAi can be used to suppress the MAELO or Δ12 fatty acid desaturase genes, resulting in accumulation of pathway intermediates. However, neither the Declaration nor specification as filed provide sufficient evidence that these results would have been considered unexpected by those of skill in the art. Paragraphs 16 and 26 provide conclusory statements that the level of accumulation of precursor DGLA could not have been predicted, and that it was unexpected that a co-suppression method could be effective in suppressing three genes encoding desaturases. However, these are conclusory statements that are not considered to be of substantial evidentiary value in the absence of objective factual evidence that others of skill would not have expected the results. Note that MPEP 716.01(III) directs the Examiner to take into consideration whether or not an expert offering an opinion has an interest in the outcome of prosecution. In this case, Declarant is an employee of the assignee of the application, and so is considered to have such an interest.

Fourth, Applicant asserts that the amount of linolenic acid produced by the Δ6 fatty acid desaturase was statistically significant and unexpected. This is unpersuasive because it is not clear, as discussed above and in more detail below, that those of skill would not have expected the results obtained by Declarant.

Applicant asserts that evidence supporting the positions that the results presented in the Declaration and the specification are unexpected, and that one of ordinary skill would not have had a reasonable expectation of success in combining the cited references, can be found in the post-filing non-patent references Proudfoot (2007) and Nakayashiki (2008).

Applicant focuses first on a passage from Proudfoot that states:

> In the fission yeast *Saccharomyces pombe,* gene silencing has been shown unexpectedly to involve the RNA interference (RNAi) pathway and in particular the RNase III enzyme Dicer.

This quote, taken out of context, might give one the impression that it was unexpected that *pombe* supported RNAi. However, when read in the context of the article as a whole it becomes apparent that Proudfoot was indicating that, at some point in the past, it was considered surprising that transcriptional gene silencing (e.g. by DNA methylation) in *pombe* involved elements of the RNAi pathway. Proudfoot did not indicate that it was surprising that RNAi existed in *pombe*. In fact, it was well known in the prior art that dsRNA-mediated gene silencing existed in *pombe* (see Raponi et al (Nucl. Acids. Res. 31(15): 4481-4489, 2003)). Furthermore, it was proven prior to the Proudfoot publication that RNAi in *pombe* also functioned by the more conventional posttranscriptional pathway of mRNA degradation (see Sigova et al (Genes Dev. 18: 2359-2367, 2004, at abstract). This references are supplied for Applicant's convenience, but are not depended on for the rejection. Thus, at the time the instant application was filed, it was no surprise that RNAi functioned in gene silencing in *pombe*.

Applicant also points out that both Proudfoot and Nakayashiki indicated that *S. cerevisiae* lacks the machinery to perform RNAi, and that Nakayashiki indicates that *U. maydis* lacks this machinery as well. Nakayashiki further states that *U. hordei*, a close relative to *U. maydis* performs RNAi, thus the loss of the RNA silencing machinery seems to sporadically occur in the fungi kingdom. The question then is whether the observation of sporadic loss of the RNA silencing machinery in the fungal kingdom is sufficient to eliminate a reasonable expectation of success in combining the cited references. In Table 1, Nakayashiki lists 13 fungal or fungal-like organisms that perform RNAi. At least 7 of these were known to perform RNAi prior to the time the instant application was filed (see references listed in the far right column of the Table). There is no evidence of record that it was known at the time of filing that either *S. cerevisiae* or *U. maydis* lacked the ability to perform RNAi. In view of the fact that RNAi was recognized to be conserved across the animal, plant, and fungal kingdoms prior to the time of the invention (Ueda (2001), above), there was no reason of record to doubt that fungi of the genus *Mortierella* could perform RNAi. Furthermore, even if those of skill in the art at the time of the invention knew that 2 unrelated species of fungi (*S. cerevisiae* and *U. maydis*) lacked the RNAi pathway, this would not have provided a substantial reason to doubt that fungi from the genus *Mortierella* lacked the RNAi pathway. This is because the pathway was known to be conserved across animal, plant, and fungal kingdoms, and in at least 7 different fungal organisms (Nakayashiki, 2008, Table 1, far right column). Thus the preponderance of evidence suggests that species that lack the capacity to perform RNAi are exceptions to the rule, and one of

ordinary skill would have had a reasonable expectation of success in combining the cited references.

With further regard to unexpected results, Applicant asserts at pages 6 and 7 of the response that the Declaration of Dr. Fukushi-Mizutani is entitled to more weight than that of a layman and that it is improper for the Office to substitute its judgement for that of an established expert. The Office has not done so. The Office has considered the teachings of the prior art and all of the evidence and opinions of record, accorded them appropriate weight, giving greater credence to evidence than opinion, and made a determination of obviousness.

Applicant asserts that the Declaration indicates that it was unexpected that RNAi cosuppression worked for all three desaturase genes, and that suppression of D6 desaturase resulted in suppression of direct or indirect products by more than 50% and a 2-fold increase in substrate concentration. However, the Declaration and response provide no specific reason that this is surprising, and no evidentiary support to indicate that others of skill in the art would have found it so. Accordingly, this is a statement of opinion, and has been treated as such. The evidence of record indicates that one of ordinary skill could have combined the cited references with a reasonable expectation of success. In view of the prior art, neither partial nor complete expression inhibition would have been considered surprising (see Ueda at pages 196, 197, and 201, and the Action of 4/7/09, paragraph bridging pages 10 and 11).

For these reasons the rejections are maintained.

Request for Interview

At page 2 of the response, Applicant set forth a request for an interview with the Examiner in the event that the application was not found to be in condition for allowance. This request was attached to an amendment which must be acted on by the Office in a timely fashion. In the future, Applicant is invited to contact the Examiner and/or his supervisor directly to arrange any interviews prior to the submission of amendments, so that any remaining issues can be discussed prior to Examiner's action.

Conclusion

No claim is allowed.

All claims are drawn to the same invention claimed in the application prior to the entry of the submission under 37 CFR 1.114 and could have been finally rejected on the grounds and art of record in the next Office action if they had been entered in the application prior to entry under 37 CFR 1.114. Accordingly, **THIS ACTION IS MADE FINAL** even though it is a first action after the filing of a request for continued examination and the submission under 37 CFR 1.114. See MPEP § 706.07(b). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any

extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner(s) should be directed to Richard Schnizer, whose telephone number is 571-272-0762. The examiner can normally be reached Monday through Friday between the hours of 6:00 AM and 3:30 PM. The examiner is off on alternate Fridays, but is sometimes in the office anyway.

If attempts to reach the examiner by telephone are unsuccessful, the Examiner's supervisor, James (Doug) Schultz, can be reached at (571) 272-0763. The official central fax number is 571-273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at 800-786-9199.

/Richard Schnizer/
Primary Examiner, Art Unit 1635